Biochemical Pharmacology, Vol. 27, pp. 1103–1108.
© Pergamon Press Ltd., 1978. Printed in Great Britain.

0006-2952/78/0415-1103$02.00/0

COMMENTARY

LIPIDOSIS INDUCED BY AMPHIPHILIC CATIONIC DRUGS

HEINZ LÜLLMANN, RENATE LÜLLMANN-RAUCH and OTMAR WASSERMANN
Departments of Pharmacology, Anatomy and Toxicology, University of Kiel, Kiel, West Germany

Many drugs are accumulated by different tissues to a remarkable degree. Expressed as tissue/medium ratios (or tissue/plasma ratios) the accumulation can amount to more than 100-fold after repeated drug administration [1]. Among others, amphiphilic compounds, which consist of a basic amine group attached over a short side chain to a hydrophobic moiety, belong to these drugs. A strong accumulation of a drug within cells presupposes that the compound in question can easily penetrate the plasmalemma thus getting access to the large surface of the intracellular membranes to which it mainly becomes adsorbed. The penetration rate will depend (a) on the pK_a of the amine group: the non-protonized form will facilitate the penetration, the protonized form will reduce it; and (b) on the degree of hydrophobicity

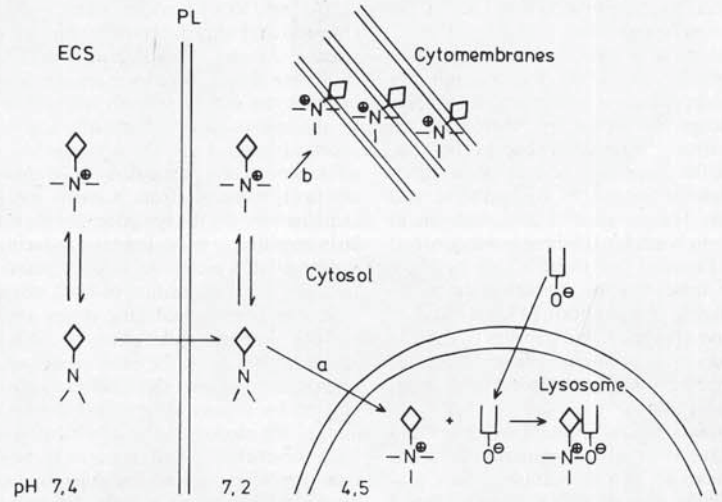
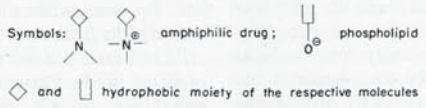


Fig. 1. Diagrammatic presentation of cellular uptake, lysosomal accumulation, and membraneous adsorption of an amphiphilic cationic drug during the initial phase of exposure. The pK_a of the amine group is considered to be above 7. After penetration through the plasmalemma (PL) the drug will be subjected to two competing processes: (1) The protonized form will become adsorbed to lipid-water interphases such as the huge surface of cytomembranes. (2) The non-protonized form will penetrate into the lysosomes and becomes trapped because of high proton concentration (pH around 4.5). Within the lysosomes, the cationic amphiphilic drug forms complexes with polar lipids. Since the complexes are removed from the diffusion equilibria, continuous gradients both for the amphiphilic drug and for the polar lipids are sustained leading to a gradual piling-up of drug-lipid complexes. The rate of lysosomal accumulation of a drug will depend, among others, upon the initial ratio between the two competing intracellular events (a), and (b). Cationic amphiphilic drugs that are not readily adsorbed (process (b) small as compared with (a)) will display particularly high initial rates of lysosomal accumulation. This may even lead to lysosomal swelling due to osmotic pressure, probably because the supply of polar lipids lags behind, which would complex with the drug and there-with remove it from the osmotic equilibrium. Chloroquine can be considered as an example for such drugs. In the case of other amphiphilic drugs that are readily adsorbed (process (b) considerably competing with process (a)) lysosomal accumulation may proceed at a relatively low rate without producing appreciable lysosomal swelling. Upon long-lasting exposure the adsorption process (b) will be saturated and will no longer compete with lysosomal accumulation (a).



of the entire molecule: if the apolarity is extremely pronounced, even a protonized amine group will not completely hinder the penetration of the molecule through the plasmalemma.

Intracellularly, an amphiphilic cationic compound will not be evenly distributed, since the drug displays varying affinities for the cellular compartments dependent upon the milieus present therein. Of particular interest is the compartment that is represented by lysosomes. They are membrane-limited vesicles distinguished by an unusually high intravesicular proton concentration (pH about 4.5) [2]. The amphiphilic amines present in the cytosol will penetrate into the lysosomes, become protonized and thus trapped in the acid milieu, as schematically depicted in Fig. 1. The intralysosomal accumulation of several amphiphilic amines and some basic dyes has been demonstrated, e.g. for chloroquine [2, 3], mepacrine [4, 5], chlorpromazine [6], chlorphentermine [7], neutral red [3, 5, 8], acridine orange [9, 10]. De Duve and coworkers [2] have coined the term "lysosomotropic drugs" for this kind of compounds. It should be mentioned that the accumulation proceeds as long as the pH of the lysosomes is kept low.

Within the lysosomes a second event takes place which is considered to play a key rôle for inducing lipid storage as observed upon treatment with amphiphilic cationic drugs. As shown by NMR-spectroscopy [11, 12] a strong interaction occurs between cationic amphiphilic drugs and certain polar lipids resulting in complexes formed by hydrophobic and electrostatic forces. The presence of phospholipids in this complexed form bears far-reaching consequences: they are protected against enzymatic attack by phospholipases, since these require the substrate to be present in negatively charged form [13, 14]. Reduction of this negative charge of lipid micelles by cations considerably reduces the enzymatic efficacy. This has been demonstrated for some lipidosis-inducing drugs by using liposomes [15, 16].

The complexation between amphiphilic drugs and polar lipids will result in an accumulation of lipids within the lysosomes by two mechanisms: (a) a gradient for polar lipids is sustained from the cytosol into the lysosome; and (b) the lipid-drug complex can no longer enzymatically be degraded. As soon as the concentration of the polar lipids has attained a certain level, they arrange themselves in a typical order giving rise to the formation of lamellated or crystalloid structures, which seem to emanate from the homogenous lysosomal matrix (Fig. 2a). With time the lamellated or crystalloid bodies grow, still possessing the limiting membrane (Fig. 2b) and acid phosphatase activity [17]. Upon further exposure or continued treatment the inclusion bodies loose their limiting membrane and the cytochemically demonstrable activity of acid phosphatase thus representing telolysosomes (Fig. 2c).

The formation of lamellated and of crystalloid bodies can be induced by amphiphilic drugs in cultured cells within several hours, as shown for example in peritoneal macrophages [17, 18] and in ganglion cells [6, 19]. The use of cultured cells is particularly suitable for studying structure–activity relationships, since such a simple system lacks complications induced by distribution phenomena and by drug metabolism. The situation *in vivo* is much more complicated: (a) the drug must be devoid of high acute toxicity to warrant doses high enough to interact with polar lipids on an approximately equimolar base; (b) a rapid biotransformation into polar metabolites greatly reduces the efficacy to induce lipidosis; (c) pharmacokinetic properties of the drugs may cause preferences for certain organs.

A list of drugs so far reported to induce lipidosis, either in cultured cells or both under *in vitro* and *in vivo* conditions, is given in Table 1. For four drugs lipidosis-like alterations have been reported to occur not only in experimental animals but also in humans.

In principle, drug-induced lipidosis is a generalized phenomenon not restricted to certain tissues. Accumulation of phospholipids has been demonstrated biochemically to occur in cultured fibroblasts [20] as well as in several organs of chronically treated animals, e.g. in lung [21–23], in liver [24–30], in kidney [27], in adrenal glands [22] and in spleen [26]. A review of the morphological findings has been given elsewhere [31]. In animals chronically treated with a potent inducer of lipidosis many organs show histochemical and ultrastructural symptoms of severe lipid storage. Among these organs are the lung, various endocrine glands, lymphatic organs, white blood cells. Hepatocytes can be severely affected, too, dependent on the species-specific metabolic fate of the drug. Of practical interest are the lipidosis-like alterations in white blood cells, particularly in lymphocytes: (a) they are easily available from humans for ultrastructural examination; (b) the lymphocytes seem to be particularly sensitive to most lipidosis-inducing drugs showing lamellated inclusions already before or concomitantly with generalization of lipid storage [32, 33].

Several lipidosis-inducing drugs affect the central nervous system, while others do not [34, 35]. This variability might be the consequence of differing drug distribution. When the central nervous system is affected by a given drug, great differences are found among the various parts and neuron types [35, 36]. Such diversities might tentatively be explained by quantitative and qualitative differences concerning the intracellular turnover of polar lipids, such that a disturbed lipid catabolism has further-reaching consequences for some types of neurons than it has for others. Most lipidosis-inducing drugs are known to cause conspicuous storage in autonomic and sensory ganglia, irrespective of their ability to affect the central nervous system.

Of particular interest are lipidosis-like alterations in ocular tissues, since two amphiphilic drugs (chloroquine, amiodarone) have become ill-famous because of their ocular side effects in humans (see below). Many lipidosis-inducing drugs have been shown in animal experiments to cause considerable lipid storage in retinal pigment epithelium and/or in sensory retina [37–40], in the cornea and in the lens which then can develop a cataract [41].

Finally, axonal and muscular changes should be mentioned, which are often associated with generalized lipidosis, while their cytological character is not entirely identical with that of the alterations in other cells and does not obviously reflect lysosomal storage of polar lipids. Particularly the preterminal and terminal parts of all kinds of peripheral axons show

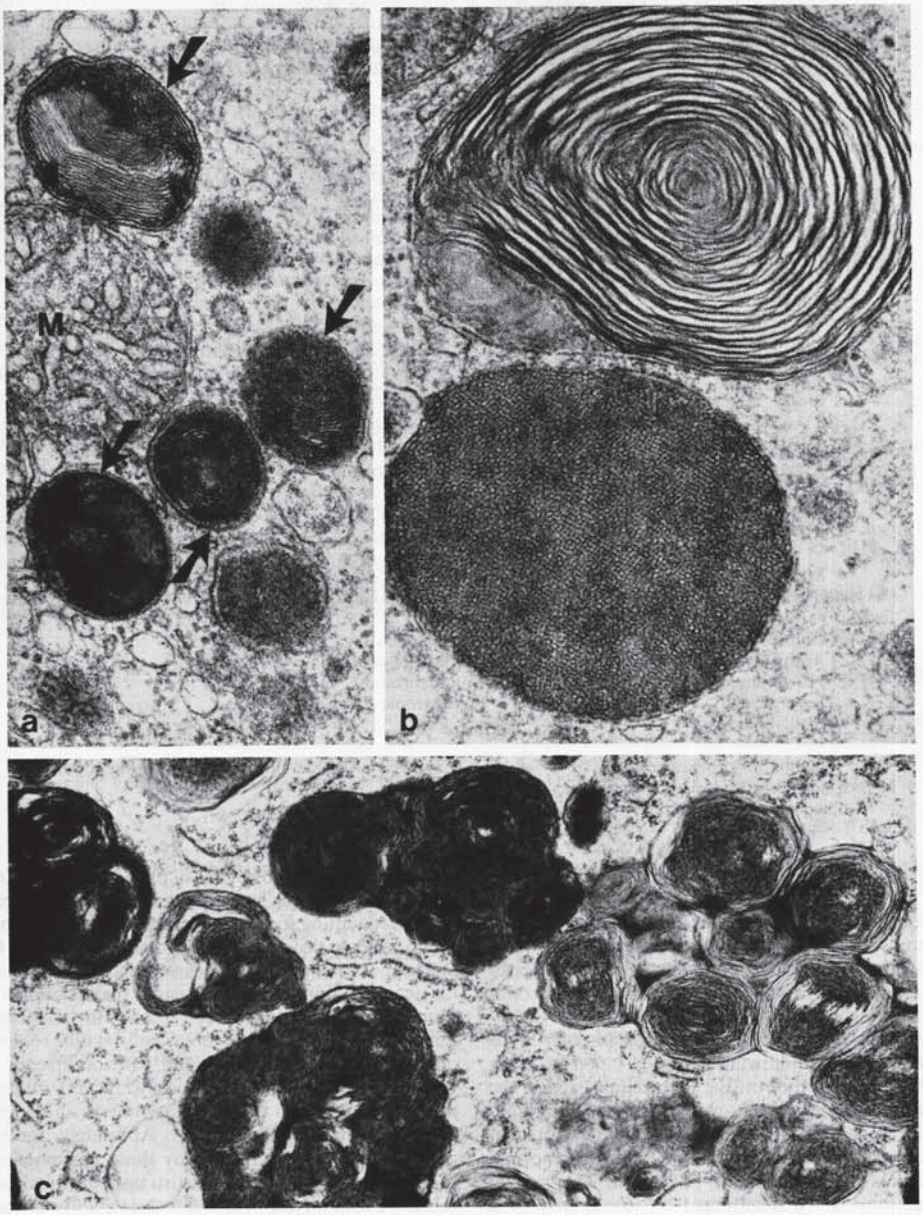


Fig. 2. Electron micrographs showing drug-induced cytoplasmic inclusion bodies. (a) Adrenocortical cell of a rat sacrificed 24 hr after a single oral application of iprindole (100 mg/kg). Arrows point to lysosomal bodies which contain lamellated material. M, mitochondrium ×61,500. (b). Inclusions with lamellated or crystalloid patterns from a chromaffin cell of a rat treated with 1-chloro-amitriptyline (120 mg/kg p.o., 10 wk) ×78,400. (c). Lamellated inclusions from a retinal ganglion cell of a rat treated with 4,4'-diethyl-aminoethoxyhexestrol (75 mg/kg p.o., 5 wk) ×28,500.

massive accumulation of polymorphous osmiophilic material [34, 42, 43]. In skeletal muscle various degenerative symptoms including fibre necrosis are seen [34, 44, 45]. Pathogenesis of this drug-induced neuro-myopathy remains to be further elucidated.

Side effects in humans. Four amphiphilic cationic drugs have so far been demonstrated to induce lipidosis-like cellular alterations in humans: chloroquine, 4,4'-diethyl-aminoethoxyhexestrol, amiodarone, and perhexiline. The clinical symptoms which have led to laboratory investigations are various: visual impairment due to retinal alterations (chloroquine, [46]) and corneal opacities (chloroquine, [47]; amiodarone, [48]), hepatosplenomegaly and abnormalities

Table 1. List of amphiphilic drugs known to induce lipidosis

(1). Inducers of generalized lipidosis as demonstrated in humans and in animals		
Chloroquine	antimalarial drug	19, 27, 34, 36–38, 47, 51, 55
4,4′-diethylamino-ethoxyhexestrol	antianginal drug	24–28, 49
Amiodarone[a]	antianginal drug	48, 56
Perhexiline	antianginal drug	50, 52, 57
(2). Inducers of generalized lipidosis as demonstrated in animals		
Imipramine	antidepressant	31, 32, 35, 39
Clomipramine	antidepressant	
Iprindole	antidepressant	
l-Chloro-amitriptyline	antidepressant	
Zimelidine	antidepressant	56
Clozapine	neuroleptic[b] drug	58
Chlorphentermine	anorectic drug	1, 21–23, 31, 33, 35, 39, 41
Fenfluramine	anorectic drug	59
Triparanol	hypocholesterolemic drug	60, 61
AY-9944	hypocholesterolemic drug	30, 40, 61
Azacosterol	hypocholesterolemic drug	61
Chlorcyclizine	antihistaminic[b] drug	62
AC-3579	tranquillizer antileucemic drug	20, 29, 63, 64
Mepacrine	antimalarial drug	7, 65
(3). Drugs with low lipidosis-inducing efficacy in whole animals but having pronounced efficacy in cultured cells		
Chlorpromazine	neuroleptic drug	6, 17, 32
Amitriptyline	antidepressant	17, 32
Noxiptiline	antidepressant	17, 32
Lysergic acid diethylamide[b]	psychotropic drug	66

[a] In humans only corneal cells have so far been demonstrated to develop lipidosis-like alterations; in rats, however, lipidosis is generalized.
[b] Studied only in cell culture.

of liver function (4,4′-diethylaminoethoxyhexestrol, [24, 25, 49]; perhexiline, [50]), neuro-myopathies (chloroquine, [51]; perhexiline, [52]). Most clinical symptoms are associated with lipidosis-like alterations in the respective organs, and in most cases these cytological changes can be reproduced in animal experiments with the respective drug.

Reversibility. In principle, the storage of polar lipids is reversible by withdrawing the drug. Upon lowering the drug concentration in the extracellular space, the diffusion gradient will be reversed and the drug-lipid complexes will slowly dissociate. The lipids will regain their normal properties and will re-enter their normal fate, namely degradation or utilization. The rate of reversibility will be determined by the affinity of a given drug towards the lipids. Excessive lipid storage may cause secondary cellular changes, the reversibility of which will depend on the regenerative abilities of the affected cell type.

Other possible mechanisms contributing to drug-induced lysosomal storage. Experiments on cultured fibroblasts have yielded evidence that chloroquine inhibits the degradation of proteins and of mucopolysaccharides [3, 53]. After chronic administration of chloroquine to whole animals, however, polar lipids seem to be the major storage material. The significance of the above findings from *in vitro* experiments for the chloroquine-induced storage syndroms seen under *in vivo* conditions remains to be elucidated.

Redirection of phospholipid biosynthesis towards acidic phospholipids has been proposed as the mechanism accounting for lipid storage induced by amphiphilic cationic drugs [54]. This may contribute to the increase of total phospholipids and may be the reason for accumulation of unusual acidic phospholipids as found in humans and in rats treated with 4,4′-diethylaminoethoxyhexestrol [24–27] or with chloroquine [27]. On the other hand, the occurrence of these nevertheless minor fractions, while very interesting under the aspect of lipid biosynthesis, should not be overestimated quantitatively. Absolutely, the largest amounts are contributed by those phospholipids that form the major fractions also under normal conditions, irrespectively of whether their relative proportions may be unchanged or even slightly lowered [27].

Conclusions

Drug-induced lipidosis can be looked upon under two aspects, (a) as a drug side effect, and (b) as a cytological phenomenon which might be a useful tool in cell biology.

(a) A causal relationship between the clinical symptoms and the associated lipidosis-like cellular alterations seen in humans (see above) appears very likely. Thus drug-induced lipidosis can indeed gain practical significance. Obviously, the therapeutic risk of applying a lipidosis-inducing drug should be balanced against the risk of the disease. In this context, it

should be mentioned that the introduction of halogen atoms into the hydrophobic moiety of drug molecules, a procedure commonly applied to alter pharmacokinetic properties, may enhance the lipidosis-inducing potency.

(b) Drug-induced lipidosis might prove to be useful for studying the cytological events of lysosomal storage of endogenous material. Furthermore, if it were possible, by means of physicochemical methods, to establish the spectrum of polar lipids preferentially interacting with a given drug, some insight into polar lipid turnover of a given cell type might be gained from the observation of absence or presence of lipid storage after chronic treatment.

Finally we should like to stress the following point. We do not believe that drug-induced lipidosis as discussed in this commentary is an appropriate experimental model for studying the inherited lipidoses of man as has been proposed [30]. According to the concept outlined above, it is a fairly unspecific alteration of substrates rather than an inhibition or a reduction of a particular enzyme that leads to intralysosomal accumulation of polar lipids. The result is, quite in contrast to the inherited storage diseases, storage of a whole spectrum of polar lipids, depending on the cell type and its peculiarities of lipid turnover, and on the affinities of the drug to different polar lipids.

REFERENCES

1. H. Lüllmann, E. Rossen and K.-U. Seiler, *J. Pharm. Pharmac.* **25,** 239 (1973).
2. C. De Duve, T. De Barsy, B. Poole, A. Trouet, P. Tulkens and F. Van Hoof, *Biochem. Pharmac.* **23,** 2495 (1974).
3. M. Wibo and B. Poole, *J. Cell Biol.* **63,** 430 (1974).
4. A. L. Bastos, A. M. Terrinha, J. D. Vigario, J. F. Moura-Nunes and J. L. Nunes-Petisca, *Expl Cell Res.* **42,** 84 (1966).
5. A. C. Allison and M. R. Young, in *Lysosomes in Biology and Pathology* (Eds J. T. Dingle and H. B. Fell) Vol. 2, p. 600. North-Holland, Amsterdam (1969).
6. C. F. Brosnan, M. B. Bunge and M. R. Murray, *J. Neuropath. exp. Neurol.* **29,** 337 (1970).
7. U. Gidion and O. Wassermann, (unpublished).
8. H. Koenig, in *Lysosomes in Biology and Pathology* (Eds J. T. Dingle and H. B. Fell) Vol. 2, p. 111. North-Holland, Amsterdam (1969).
9. E. Robbins and P. I. Marcus, *J. Cell Biol.* **18,** 237 (1963).
10. E. Robbins, P. I. Marcus and N. K. Gonatas, *J. Cell Biol.* **21,** 49 (1964).
11. J. K. Seydel and O. Wassermann, *Naunyn-Schmiedeberg's Arch. Pharmac.* **279,** 207 (1973).
12. J. K. Seydel and O. Wassermann, *Biochem. Pharmac.* **25,** 2357 (1976).
13. R. M. C. Dawson and A. D. Bangham, *Biochem. J.* **72,** 493 (1959).
14. R. M. C. Dawson in *Form and Function of Phospholipids* (Eds G. B. Ansell, J. N. Hawthorne and R. M. C. Dawson), p. 97. Elsevier, Amsterdam (1973).
15. H. Schwarting, K.-U. Seiler and O. Wassermann, *Naunyn-Schmiedeberg's Arch. Pharmak. p.* 293 (*Suppl.*), R 57 (1976).
16. D. Rathje, M. D. Thesis, Kiel (1977).
17. D. Drenckhahn, L. Kleine and R. Lüllmann-Rauch, *Lab. Invest.* **35,** 116 (1976).
18. D. Drenckhahn, L. Kleine and H. J. Lepthin, *Anat. Anz.* (Suppl.) in press (1977).
19. K. Tischner, *Acta Neuropath.* **33,** 23 (1975).
20. M. Philippart and E. Kamensky, in *Current Trends in Sphingolipidoses and Allied Disorders* (Eds B. W. Volk and L. Schneck), p. 473. Plenum Publ. Corp., New York (1976).
21. R. Schmien, K.-U. Seiler and O. Wassermann, *Naunyn-Schmiedeberg's Arch. Pharmak.* **283,** 331 (1974).
22. K.-U. Seiler and O. Wassermann, *Naunyn-Schmiedeberg's Arch. Pharmak.* **288,** 261 (1975).
23. D. Karabelnik and G. Zbinden, *Hoppe-Seyler's Z. physiol. Chem.* **356,** 1151 (1975).
24. A. Yamamoto, S. Adachi, T. Ishibe, Y. Shinji, Y. Kaki-Uchi, K. Seki and T. Kitani, *Lipids* **5,** 566 (1970).
25. A. Yamamoto, S. Adachi, K. Ishikawa, T. Yokomura, T. Kitani, T. Nasu, T. Imoto and M. Nishikawa, *J. Biochem., Tokyo* **70,** 775 (1971).
26. S. Adachi, Y. Matsuzawa, T. Yokomura, K. Ishikawa, S. Uhara, A. Yamamoto and M. Nishikawa, *Lipids* **7,** 1 (1972).
27. A. Yamamoto, S. Adachi, Y. Matsuzawa, T. Kitani, A. Hiraoka and K. Seki, *Lipids* **11,** 616 (1976).
28. F. A. De La Iglesia, G. Feuer, J. E. McGuire and A. Takada, *Toxic. appl. Pharmac.* **34,** 28 (1975).
29. J. Hildebrand, O. Thys and Y. Gerin, *Lab. Invest.* **28,** 83 (1973).
30. N. Sakuragawa, M. Sakuragawa, T. Kuwabara, P. G. Pentchev, J. A. Barranger and R. O. Brady, *Science, N.Y.* **196,** 317 (1977).
31. H. Lüllmann, R. Lüllmann-Rauch and O. Wassermann, *CRC Critical Rev. Toxic.* **4,** 185 (1975).
32. R. Lüllmann-Rauch, *Naunyn-Schmiedeberg's Arch. Pharmak.* **286,** 165 (1974).
33. R. Lüllmann-Rauch, *Toxic. appl. Pharmac.* **32,** 32 (1975).
34. G. W. Klinghardt, *Acta neuropath.* **28,** 117 (1974).
35. R. Lüllmann-Rauch, *Acta neuropath.* **29,** 237 (1974).
39. R. Lüllmann-Rauch, *Acta neuropath.* **35,** 55 (1976).
37. R. Abraham and R. J. Hendy, *Exp. Molec. Path.* **12,** 185 (1970).
38. M. H. Gregory, D. A. Rutty and R. D. Wood, *J. Path. Bact.* **102,** 139 (1970).
39. R. Lüllmann-Rauch, *Acta neuropath.* **35,** 55 (1976).
40. M. Sakuragawa, *Invest. Ophthalmol.* **15,** 1022 (1976).
41. D. Drenckhahn and R. Lüllmann-Rauch, *Exp. Eye Res.* **24,** 621 (1977).
42. D. Drenckhahn and R. Lüllmann-Rauch, *Cell Tiss. Res.* **171,** 273 (1976).
43. D. Drenckhahn, *Virchows Arch. B. Cell Path.* **23,** 87 (1977).
44. R. D. Macdonald and A. G. Engel, *J. Neuropath. exp. Neurol.* **29,** 479 (1970).
45. D. Drenckhahn and R. Lüllmann-Rauch, *Virchows Arch. B Cell Path.* **20,** 343 (1976).
46. M. S. Ramsey and B. S. Fine, *Am. J. Ophthalmol.* **73,** 229 (1972).
47. H. J. Thiel and G. Pülhorn, *Klin. Mbl. Augenheilk.* **166,** 791 (1975).
48. D. Toussaint and S. Pohl, *Bull. Soc. belg. Ophthal.* **153,** 675 (1969).
49. T. Shikata, T. Kanetaka, Y. Endo and K. Nagashima, *Acta path. jap.* **22,** 517 (1972).
50. A. Lageron, R. Poupon, P. P. De Saint-Maur and V. G. Levy, *Lancet* **1,** 483 (1977).
51. R. Garcin, P. Rondot and M. Fardeau, *Rev. Neurol.* 177 (1964).
52. J. M. Mussini, J. J. Hauw and R. Escourolle, *Acta neuropath.* **38,** 53 (1977).
53. S. O. Lie and B. Schofield, *Biochem. Pharmac.* **22,** 3109 (1973).
54. R. H. Michell, D. Allan, M. Bowley and D. N. Brindley, *J. Pharm. Pharmac.* **28,** 331 (1976).
55. R. Abraham and R. Hendy, *Exp. molec. Pathol.* **12,** 148 (1970).

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.